AD NUMBER

AD834635

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; JAN 1967. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Releases Branch, Frederick, MD 21701.

AUTHORITY

Fort Detrick/SMUFD ltr dtd 15 Feb 1972

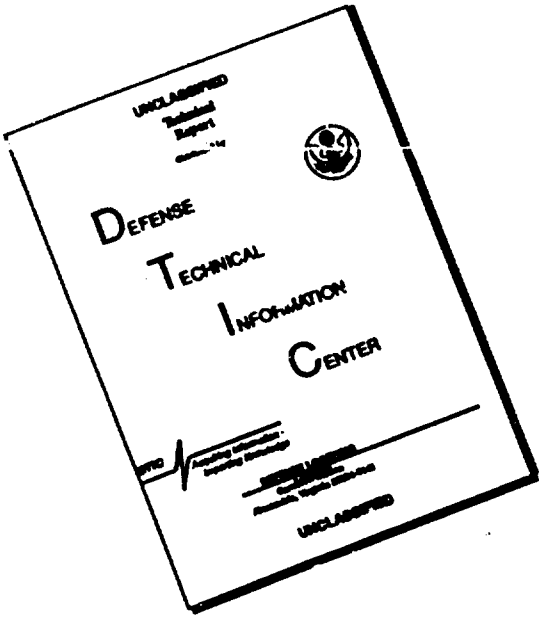
TRANSLATION NO. 1899

DATE: 5 JANUARY 1967

AD834635

21701

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

INCREASED RESISTANCE TO INFECTION AFTER ENDOTOXIN INJECTION IN CONVENTIONAL AND AXENIC YOUNG MICE

Comptes Rendus Academie
de Science, Paris
(Proceedings of the Academy
of Science, Paris)
262: 1914-17, 1966

Monique Parant and
Edmond Sacquet
introduced by
Jacques Trefouel

Abstract: A preliminary injection of endotoxin increased the resistance to infection in young mice. This treatment stimulated phagocytosis and the destruction of virulent bacteria. In young mice the effects were less pronounced and of shorter duration than in adult mice.

A preliminary injection of a very low concentration of endotoxin into adult mice increased their ability to eliminate and destroy virulent bacteria. It enabled the animals, infected with Klebsiella pneumoniae, to survive for a few days(1). We have studied this phenomenon with conventional and axenic mice at birth, i.e. under conditions in which active antibodies can be ruled out.

Klebsiella pneumoniae strain Caroli was injected intravenously. Techniques for counting bacteria labelled with ^{51}Cr and the measurement of radioactivity have been already described(1,2,3). Salmonella enteritidis endotoxin, extracted by the method of Boivin, was injected intraperitoneally into young mice and intravenously into adult mice 24 hours before the inoculation with the bacteria. A saturated solution of sulfadiazine (ca. 0.15 mg/ml) was injected subcutaneously into new-born mice. All injections into new-born mice were 0.05 ml in volume. The conventional mice were from the Swiss or C3H strain; the axenic mice were from the C3H/Jax strain, raised at the C.N.R.S.(4).

TABLE 1

Age* Days	Treatment	Duration of the Experiment	Number of Mice	Weight grams	Rate of Blood %	Radioactivity Liver %	Spleen %
2	none	30 min	8	1.75	59.4	17.8	2.3
	1 mcg endotoxin	" "	9	1.58	43.7**	36.6**	0.5
	10 mcg "	" "	4	1.55	37.4**	26.0**	0.4
2	none	60 "	19	1.77	49.3	29.3	2.7
	1 mcg endotoxin	" "	10	1.26	28.5**	55.9**	0.7
35	none	30 "	5	19	58.3	13.7	11.1
	1 mcg endotoxin	" "	5	22	12.6**	53.6**	13.7

* Conventional new-born mice received $5x10^6$ radioactive bacteria; adult mice received $5x10^6$ per gram.

** Statistically highly significant results by the F-test.

1. ELIMINATION OF RADIOACTIVE BACTERIA IN CONVENTIONAL MICE. After injecting heated, ^{51}Cr-labelled bacteria, the difference in radioactivity found in the blood and liver provided an estimate for the rate of elimination. Data of Table 1 show that the hepatic fixation of bacteria was increased in 2-day old mice by the preliminary injection of endotoxin. This action was less pronounced in adult mice and was in agreement with the relative weight of the liver (4.8% of the body weight after 2 days, 6.4% after 5 weeks).

On the contrary, the rate of radioactivity recovered from the spleen of young mice was lowered by the endotoxin injection. Analogous results were obtained with living bacteria (Tables 2 and 3) and, undoubtedly can be attributed to the cytotoxic action of bacterial antigen(5).

2. ELIMINATION OF LIVING BACTERIA. a. Conventional mice. One hour after inoculation, the distribution of the viable bacteria was comparable with that in new-born mice and with that in adult mice inoculated with radioactive bacteria. Three hours after inoculation, the total number of bacteria was ten times higher in the infected mice than in endotoxin-treated mice and constituted about 4.5 generations of bacteria which were almost entirely in the blood (Table 2).

TABLE 2

Age* Days	Treatment	Duration of the Experiment hours	Number of Mice	Average Number of Bacteria Blood	Liver	Spleen	Total
2	none	1	13	5,010	870	80	5,960
	1 mcg endotoxin	1	17	1,735	2,160	35	3,930
	none	3	6	75,350	8,260	-	83,610
	1 mcg endotoxin	3	6	4,460	4,400	-	8,860
40	none	1	12	84,470	10,010	16,370	110,850
	1 mcg endotoxin	1	12	32,540	26,320	27,200	86,060

* Conventional new-born mice received 4×10^3 bacteria; adult mice received 10^5.

b. Axenic mice. Numbers of bacteria shown in Table 3 were obtained from mice of different ages. Those from mice four days old or older were comparable in number and distribution with those obtained from conventional mice 2-40 days old (see Table 2). The number of bacteria was always lower in the blood of mice which had received endotoxin.

On the contrary, in two-day old mice, three hours after being inoculated with bacteria, the action of the endotoxin became noticeable. During the first hour, the elimination of the bacteria from the blood was not accelerated by the preliminary injection of endotoxin. Later on, in axenic mice, the distribution of the bacteria was different from that observed in the conventional mice, because the number of bacteria recovered from the liver was always higher.

3. RESISTANCE OF CONVENTIONAL MICE TO INFECTION. a. Survival. New-born mice were infected on the second day with 2×10^2 bacteria. The protective dose was an injection of 0.1 or 1.0 mcg of endotoxin which was much more transient than in adult mice. Although all of the test mice (53/53) died after 18 hours, those injected with endotoxin did not die until 20-48 hours (52/95 at 30 hours), depending on the size of the inoculum.

b. Numbers of bacteria in conventional mice treated with sulfadiazine. The destruction of Klebsiella pneumoniae was demonstrated by the administration of the bacteriostatic drug to adult mice which were injected with endotoxin(1). Sulfadiazine was administered to mice in doses of 7.5 mcg each time, as a control and two hours before infection, followed by one to three supplementary injections during the experiment.

The treatment slowed down bacterial multiplication, but was insufficient to stop it completely. Once again, the number of bacteria in the treated mice was the same at 24 hours as that in untreated mice at 6 hours and constituted about 11 generations of bacteria (Table 4). Under these conditions, mice which had received a protective dose of endotoxin had at 24 hours about 100 times fewer bacteria than endotoxin-free mice and there was no increase in numbers between 6 and 24 hours.

TABLE 3

Age* Days	Treatment 24 hrs before Infection	Duration of the Experiment hours	Number of Mice	Average Number of Bacteria Blood	Liver	Spleen	Total
2	none	1	21	3,800	4,340	20	8,160
	1 mcg endotoxin	1	18	4,290	3,400	0	7,690
2	none	3	6	68,500	3,060	190	71,750
	1 mcg endotoxin	3	7	30,250	8,930	0	39,180
4	none	1	5	6,580	4,495	680	11,755
	1 mcg endotoxin	1	6	910	5,930	120	6,960
8	none	1	12	6,790	4,760	1,230	12,780
	1 mcg endotoxin	1	13	395	5,940	345	6,680
45	none	1	6	142,950	37,610	32,980	213,540
	1 mcg endotoxin	1	6	49,150	36,400	37,500	123,050

*Axenic young mice received 8x10³ bacteria; adult mice received 10⁵.

TABLE 4

Treatment	Hours*	Number of Mice	Average Number of Bacteria Blood %	Liver %	Spleen %	Total &
None	6	6	76	22.6	1.4	10,076,000
Sulfadiazine	6	10	60.8	38.7	0.5	234,500
	16	6	70.1	29.7	0.2	2,965,100
	24	9	14.8	75.3	7.9	13,705,000
1 mcg Endotoxin	6	6	67.5	31.8	0.7	1,086,000
1 mcg Endotoxin	6	6	65.5	33.5	1.0	107,050
plus Sulfadiazine	16	6	11.4	86.8	1.8	43,450
	24	10	14.7	82.3	3.0	139,900

*Hours after injecting 4x10³ bacteria into two-day old mice.

CONCLUSIONS. Reticulo-endothelial cells of new-born mice show phagocytosis against carbon particles or radioactive bacteria(6.7), although the mice are very susceptible to infections at that age. Elsewhere, Sterzl observed the resistance of five-day old rats was increased by endotoxin(8). In the mouse, our results indicated elimination of Klebsiella pneumoniae from the blood could be accelerated by injecting a weak dose of endotoxin several hours after birth. The stimulation was weaker in axenic new-born mice, but became noticeable three hours after infection with bacteria. Axenic adult mice reacted like conventional adult mice to the endotoxin injection, already reported by others(9.10).

Survival and enumeration experiments in conventional sulfamide-treated mice indicated the action of endotoxin was less pronounced and of shorter duration in young mice than in adult mice(1) and there was a clearer indication that the resistance of the new-born animal is weak. These results were obtained under experimental conditions which excluded the formation of antibodies and which favored nonspecific activity. Preliminary experiments indicated it was equally possible to make young mice as tolerant to endotoxin as was the case with adult mice(2).

(*) Meeting of 13 April 1966.
(1) M. Parant, F. Boyer, L. Chedid, Comptes rendus, 260:2630 and 3218, 1965.
(2) F. Parant, M. Parant, H. Charlier, E. Sacquet, L. Chedid, Ann. Inst. Pasteur, 110, suppl. 3:198, 1966.
(3) L. Chedid, M. Parant, F. Boyer, R. C. Skarnes, Symposium on Bacterial Endotoxins, Rutgers Univ., p. 500, 1964.
(4) E. Sacquet and H. Charlier, Ann. Inst. Pasteur, 108:353, 1965.
(5) P. A. Ward, A. G. Johnson, M. R. Abell, J. exp. med. 109:463, 1959.
(6) C. Stiffel, G. Biozzi, B. Benacerraf, C. R. Soc. Biol. 150:1075, 1956.
(7) P. C. Reade and C. R. Jenkin, Immunology 9:53 and 67, 1965.
(8) J. Sterzl, M. Holub, I. Miller, Folia Microbiol. 6:289, 1961.
(9) G. J. Thorbecke and B. Benacerraf, Ann. N. Y. Acad. Sci. 78:347, 1959.
(10) M. Landy, J. L. Whitby, J. G. Michael, M. W. Woods, W. L. Newton, Proc. soc. exp. biol. 109:352, 1962.

(Pasteur Institute, 28 Rue du Docteur Roux, Paris 15e, and Center for the Selection of Laboratory Animals, Gif-sur-Yvette, Essonne).